

Thermal Tolerances of Three Tramp Ant Species (Hymenoptera: Formicidae)

by

Daniel Russ Solis & Odair Correa Bueno

ABSTRACT

Tramp ant species present a set of adaptations to their urban habitats, and there is a paucity of knowledge about how they interact with abiotic factors, like temperature. Temperature is well known to interfere with insect activity. The present study evaluated the temperature tolerance of three important tramp ant species: *Monomorium floricola* (Jerdon), *Monomorium pharaonis* (Linnaeus) and *Tetramorium bicarinatum* (Nylander). Tested temperatures were 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48 and 50°C. Ten repetitions with 20 workers each were done with each temperature and analyzed species. The number of dead workers was recorded every hour over a total of 8 hours. All procedures were done using thermal incubators at relative humidity within 50-95%. Workers of *M. pharaonis* proved more tolerant to high temperatures (30-50°C) than workers of *M. floricola* and *T. bicarinatum*. The higher the temperatures tested, greater was the recorded ant mortality, with temperature 50°C being fatal to all species after 1h of exposition. The least tolerant species to temperatures below 20°C was *T. bicarinatum*. Low temperatures tested were not fatal to any of the tested species.

Key words: Myrmicinae, *Monomorium floricola*, *Monomorium pharaonis*, *Tetramorium bicarinatum*.

INTRODUCTION

Both biotic and abiotic factors exert great influence over the functionality of organisms. Changes in these factors can be recurrent or intermittent, predictable or unpredictable, and each species differ in its ability to handle and adapt to a change of conditions (Linksvayer & Janssen 2008). According with the same authors, examples of abiotic factors would include range of

temperature and humidity, fires, droughts, hurricanes and climate changes. Temperature is an important factor driving insect activity; the body temperature of insects reflect surrounding environmental conditions, and most of their internal heat is metabolically produced, particularly by wing muscles (Chapman 1998).

The urban ant fauna presents considerable medical and economic importance, as many species are pests of domiciliary, industrial, and commercial establishments, including some which can cause relevant structural damage; many ant species are serious pests of crops and natural habitats, and some are important mechanical vectors of pathogenic microorganisms in hospital areas, and can cause severe allergic reactions (Vega & Rust 2001; Campos-Farinha *et al.* 2002; Klotz *et al.* 2008). Among urban ants, there are those species baptized 'tramp species', which feature a set of advantageous characteristics that enabled them to adapt to humanized environments, as described in Passera (1994). Tramp ant species typically have a global geographical distribution, being only absent in the coldest world parts (e.g., Antarctica) (Wetterer 2008, 2009a, 2009b, 2009c, 2009d, 2010a, 2010b).

Based on the classification of ants into functional groups proposed by Andersen (2000), it can be seen that the most common tramp ant species would include representative species from dominant Dolichoderinae (e.g., *Linepithema*), Opportunists (e.g., *Tetramorium* and *Paratrechina*) and generalized Myrmicinae (e.g., *Monomorium* and *Pheidole*). These functional groups vary in the way they respond to environmental stress and disturbances: generalized Myrmicinae are better adapted to hot climates and shaded areas, while being only moderately sensitive to low temperature; Opportunistic species are well adapted to cool, shaded, and open, warm habitats (Andersen 1995).

In spite of the importance of urban ants, little is known about their adaptability to abiotic factors at species level. For instance, a recent study with *Atta sexdens* (Linnaeus) (Angilletta *et al.* 2007) compared the temperature tolerance of workers from urban and rural regions, and found that workers from colonies in urban environments can tolerate higher temperatures (42°C) for a longer time period than workers deriving from colonies in the countryside. However, the authors could not determine whether the differences in thermal tolerance derived of some genetic component or was the result of a more flexible response to the environmental conditions.

The present study aimed at assessing the thermal tolerance of three tramp ant species: *Monomorium floricola* (Jerdon), *Monomorium pharaonis* (Linnaeus), and *Tetramorium bicarinatum* (Nylander). We expect the finds to have applicable implications to managing these pest species in the urban environment, as knowledge about such basic biology parameters of pest species can elucidate how they adapted to the urban environment.

MATERIAL AND METHODS

Maintenance of the ant colonies in the laboratory

Laboratory colonies of the ants *M. floricola*, *M. pharaonis*, and *T. bicarinatum* were held in a climatized room with controlled temperature (23-27°C) and humidity (50-80%). The colonies were brought up inside wooden boxes (10.5 cm x 8.5 cm x 1.0 cm), or test tubes (25.0 cm x 2.5 cm), the tubes containing a piece of cotton soaked in water at the distal end and were lined with red cellophane. Ants were fed with insects, larvae of *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae) and *Zophobas* sp. (Coleoptera: Tenebrionidae), adults of *Gryllus* sp. (Orthoptera: Gryllidae), and sugary liquids (water solution of honey and inverted sucrose), and pieces of sausage and sardine. Food items were presented three times a week, tap water was offered 'ad libitum'.

Assembly and analysis of the experiment

Randomly-picked workers from several colonies were exposed to the following temperatures: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48 and 50°C. Ten repetitions with 20 workers each were done with each temperature and analyzed species. These experiments were made inside a thermal incubator (Eletrolab, Model EL 101/3) containing plastic trays with water to keep interior relative humidity within 50-95%; this being the range considered adequate for rearing these ants in the laboratory, as previously mentioned. Temperature variation inside the incubator was set to a minimum of $\pm 0.5^\circ\text{C}$. In all cases, ant workers were kept in test tubes (25 cm x 2.5 cm) closed with perforated tissue as a screen. With the only exception of the temperature 25°C, the tested workers were acclimatized to the exposure temperature tests: they were brought from the rearing room (23-27°C) to a thermal incubator at 25°C, and the temperature was slowly adjusted about 0.5°C/min to the experiment temperature.

The number of dead workers was recorded every hour over a total of 8 hours. According with Hebling-Beraldo (1978), ants are immobilized at lower temperatures due to some cold narcosis, thus when dealing with temperatures below 8°C we waited for 30 min at room temperature until living ants recovered at ambient temperature (18-21°C).

Statistical analysis

Resulting percentage of surviving workers from each temperature were transformed by arcsine to yield normal distribution. Transformed data were then submitted to analysis of variance (One-way ANOVA) followed by Dunnett's test, in order to check from which temperature mortality of workers became significant over the analyzed time periods tested. Control temperature set for Dunnett's test was 25°C. Moreover, we calculated LT_{50} (lethal temperature for 50% of workers) and TL_{90} (lethal temperature for 90% of workers) for each species and period of exposure using Probit analysis. Obtained lethal temperature values were subjected to analysis of variance (Two-way ANOVA) followed by Tukey's test, comparing among different species and periods of exposure.

RESULTS

From analyzing the results of the 23 tested temperatures, it can be seen that total mortality was shared by the three species at the temperature of 50°C upon the first hour of exposure. For the other exposure periods at temperatures over 25°C, complete mortality is shown in Fig. 2. However at temperatures below 25°C, total mortality never occurred (Fig. 1).

From analyzing Tables 1, 2, and 3, it can be observed that from temperatures above 25°C at longer periods of exposure there is a decrease in the temperature value at which worker mortality becomes significant, demonstrating how workers are less tolerant to high temperatures at longer periods of exposure. Most tolerant species to high temperatures was *M. pharaonis*, with the other two presenting the same amount of tolerance.

From analyzing Table 1, which presents results for temperatures below 25°C, it can be seen that only *T. bicarinatum* exhibited significant mortality at the longer periods of exposure of 6, 7, and 8 hours. However, mortality at these temperatures with *M. floricola* and *M. pharaonis* was not significant,

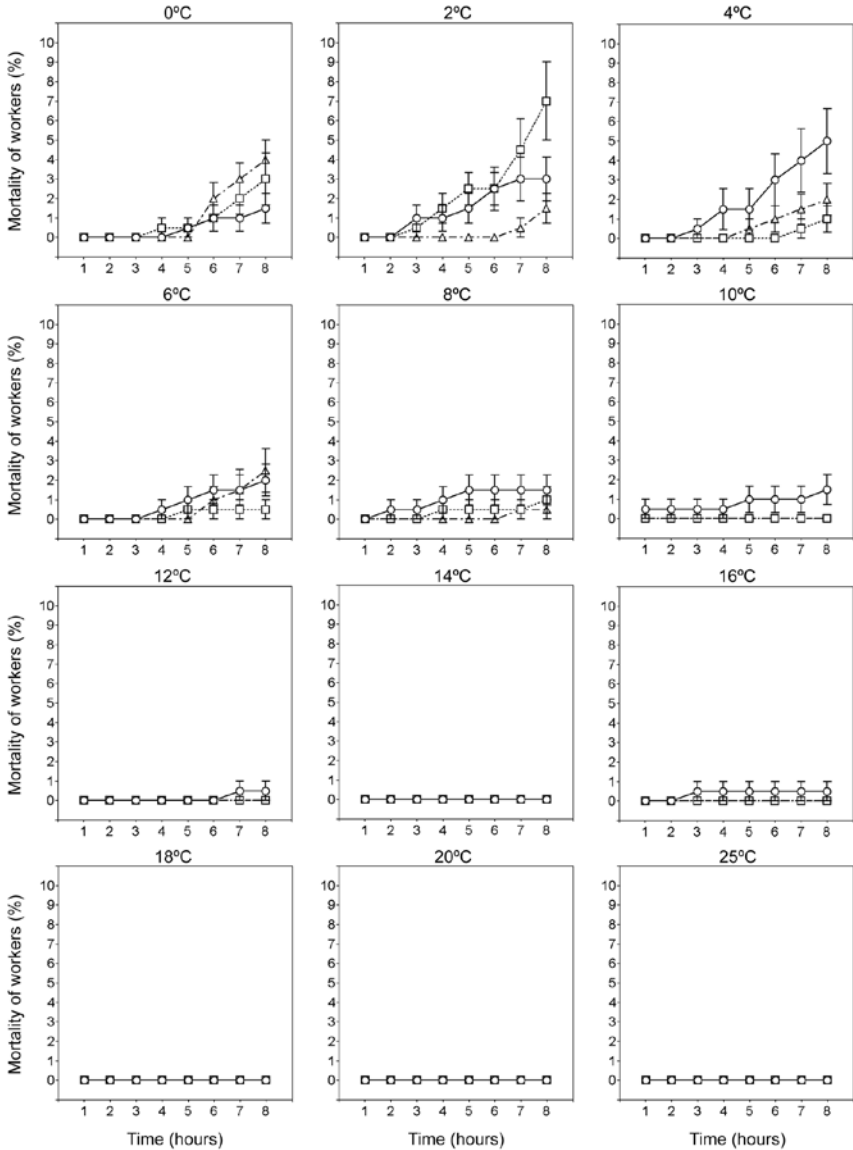


Fig. 1. Worker mortality of the tramp ant species *Monomorium floricola* (\circ), *Monomorium pharaonis* (\square), and *Tetramorium bicarinatum* (Δ) when exposed to different temperatures below 25°C over eight hours of exposure. Error bars display standard error of the mean.

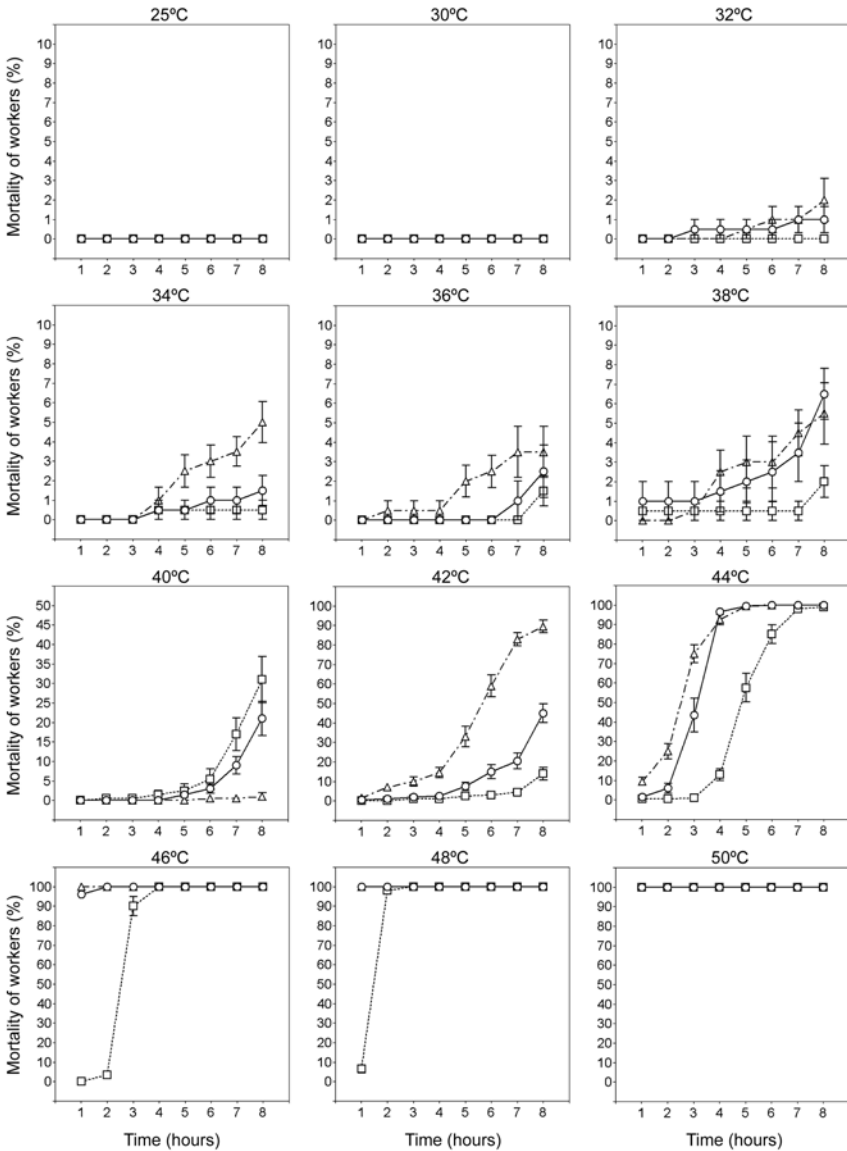


Fig. 2. Worker mortality of the tramp ant species *Monomorium floricola* (○), *Monomorium pharaonis* (□), and *Tetramorium bicarinatum* (Δ) when exposed to different temperatures above 25°C over eight hours of exposure. Error bars display standard error of the mean.

Table 1. Temperatures from which worker mortality in three tramp ant species was significant when exposed to different experimental temperatures, according with Dunnett's test ($p < 0.01$) employing 25°C as control temperature.

Species	Time (h) / Temperature (°C)							
	1	2	3	4	5	6	7	8
<i>Monomorium floricola</i>	46	44	44	44	42	42	40	38
<i>Monomorium pharaonis</i>	48	46	46	44	44	44	40	40
<i>Tetramorium bicarinatum</i>	44	42	42	42	42	0;42	0;42	0;42

Table 2. LT_{50} (Lethal Temperature to 50% of sample) obtained for the tramp ants species over eight hours of continuous exposition to different temperatures above 25°C.

Species ¹	Time (h) / Temperature (°C) ²							
	1 ^a	2 ^{ac}	3 ^{acd}	4 ^{bc}	5 ^{bd}	6 ^{bd}	7 ^{bd}	8 ^{bd}
<i>Monomorium floricola</i> ^a	45	44.8	44.1	43	42.7	42.5	42.3	41.5
<i>Monomorium pharaonis</i> ^b	48.8	46.9	45.2	44.6	43.7	43.1	42.7	42
<i>Tetramorium bicarinatum</i> ^a	44.6	44.3	43.3	42.8	42.2	41.6	41.1	40.9

Notes. ¹Figures on the same column followed by same letter did not differ by Tukey's test ($p < 0.01$). ²Figures on the same line followed by same letter did not differ by Tukey's test ($p < 0.01$).

Table 3. LT_{90} (Lethal Temperature to 90% of sample) obtained for the tramp ants species over eight hours of continuous exposition to different temperatures above 25°C.

Species ¹	Time (h) / Temperature (°C) ²							
	1 ^a	2 ^{ac}	3 ^{ac}	4 ^{ac}	5 ^{ac}	6 ^{bc}	7 ^{bc}	8 ^{bc}
<i>Monomorium floricola</i> ^a	45.9	45.6	45.4	44	44	44	44.3	44
<i>Monomorium pharaonis</i> ^b	49.6	47.8	46.1	45.8	45.2	44.5	44.2	44.5
<i>Tetramorium bicarinatum</i> ^a	45.3	45.7	44.7	44.3	44.2	43.7	43.2	43.2

Notes. ¹Figures on the same column followed by same letter did not differ by Tukey's test ($p < 0.01$). ²Figures on the same line followed by same letter did not differ by Tukey's test ($p < 0.01$).

suggesting that these species are more tolerant to lower temperatures than *T. bicarinatum*.

DISCUSSION

Hebling-Beraldo (1978) determined the LT_{50} over six hours of exposure of the Brazilian ant's *A. sexdens* and *Atta laevigata* (Smith), and found their respective tolerance limits to be 2.8°C and 36.3°C, and 1.5°C and 37.5°C. From comparing their obtained tolerance limits with our results, it becomes clear that our tested species have a broader temperature tolerance. We think this might be correlated with the fact that they are exotic invasive species, which are expected to be more tolerant to altered abiotic conditions. As an

example of a Brazilian ant invasive to other countries, Xu *et al.* (2009) obtained the LT_{50} for the fire ant *Solenopsis invicta* (Buren) of 43.5°C after one hour of exposure, which is still lower than the herein recorded with other three species. Tramp ant species have worldwide distribution and are typical of humanized areas, thus probably display physiological and behavioral adaptations that allow their survival in stressful environments wherein the native species cannot thrive (Passera 1994). For instance, some species do not build their nests in an organized fashion into the soil (herein including tramp ant species), and living under rocks and fallen branches, there are physiologically less vulnerable to lower humidity or higher temperatures (Hölldobler & Wilson 1990).

However, Linksvayer & Janssen (2008) stated opportunistic ant species are more tolerant to stressful and disturbed environments, but cannot out compete species adapted to extreme temperatures. For instance, Walters & Mackay (2004) compared heat tolerance of *Linepithema humile* (Mayr) (an invasive species) in Australia in comparison with two native species: *Iridomyrmex rufoniger* (Lown) and *Rhytidoponera convex* (Mayr). These authors found all *L. humile* died after one hour of exposure to 50°C, while others were more tolerant: total annihilation with *R. convex* only occurred after 2h of exposure, while *I. rufoniger* still presented a 50% survival after 3h of exposure to 50°C. In a similar study, Holway *et al.* (2002) compared *L. humile* with five species native to California and also found that *L. humile* was less tolerant to higher temperatures (all killed after 1h at 46°C) than native species (others were only annihilated at temperatures above 50°C).

On the other hand, Walters & Mackay (2004) advised caution when interpreting such results as apparently ants can modulate behavior in response to environmental changes to avoid exposure to extreme conditions, and temperature should not be regarded isolated from other important abiotic factors. For instance, ant species living in deserts and other dry habitats can adapt by building their nests deeper into the soil, and diurnal species will usually forage in the morning and at nightfall (Hölldobler & Wilson 1990). In a study of diurnal foraging in three tramp ant species from the Galapagos Islands, Meier (1994) found that foraging workers of *M. floricola* have a pattern of activity over the day, with peak activity during the day (from 06:00h

to 18:00h). From analyzing the foraging pattern for *M. floricola* presented by Meier (1994) on page 56, two significant decreases in the foraging at the peaks of activity can be perceived: between 11:00h and 13:00h and from 16:00h to 18:00h. Upon comparing Meier's graph with another illustration on page 56 of Meier (1994) presenting temperature variation over the day, it can be seen that the two reductions in foraging coincided with temperature variations: the first foraging reduction can be related with the peak temperatures of the day 30-32°C while the second reduction can be related with temperature dropping below 26°C. Foraging ceased completely between 00:00h and 05:00h, period at which mean temperature remained around 22°C. Interestingly, worker mortality was null in our experiments within the temperature range 18-30°C, illustrating that *M. floricola* is well adapted to this temperature range. It should be emphasized, however, that even if a given ant species is tolerant to higher temperatures, this does not mean it will forage at such temperatures (Cerda *et al.* 1998).

Cerda *et al.* (1998) evaluated the thermal tolerance of a Spanish ant community of 11 species. One of their conclusions revealed a direct correlation between worker size and maximum critical temperature (i.e., a 10-min exposure to a given temperature in which 50% of worker wither died or were severely impaired): species with larger workers were more tolerant to higher temperatures. In our experiments, *T. bicarinatum* was the largest species and proved the least tolerant.

Francke *et al.* (1985) hypothesized that tolerance to different temperatures in ants can be influenced by worker age, ambient humidity, previous thermal regime, and colony diet, apart from colony location (Angilletta *et al.* 2007). As all three species were reared under the same room conditions and the tests employed only workers found outside the colonies - which are regarded as the oldest (Hölldobler & Wilson 1990) - we think the observed differences between species directly reflect their intrinsic temperature tolerances.

Based on the results of this study we can state that workers of *M. pharaonis* are the most tolerant to higher temperatures among the tested species. Regarding temperatures below 25°C, *T. bicarinatum* proved also the least tolerant. Other temperature studies with these species ought to evaluate their regulatory capacity at colony level, thus assessing general survival develop-

ment of colony members, and the correlation of temperature with other environmental variables (like humidity), and the existing behavioral and physiological mechanisms to cope with extreme conditions.

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